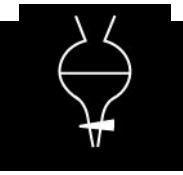
MOLECULAR IMPRINTS FOR SOLID-PHASE EXTRACTION



P. A. G. Cormack, University of Strathclyde, Glasgow, UK
K. Haupt, INTS-INSERM, Paris, France

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Among the most important fields in analytical chemistry are medical, food and environmental analysis, where the target analytes are often present at very low concentrations in rather complex matrices. Methods currently used for analysis, such as liquid chromatography, gas chromatography or capillary electrophoresis, must therefore be preceded by a selective isolation and concentration step. Commonly used techniques for the extraction and clean up of analytes from environmental and biological samples are liquid–liquid extraction (LLE) and solid-phase extraction (SPE). Supercritical-fluid extraction (SFE) has also attracted interest, although it has not found widespread application thus far because of the specialized equipment required. The advantages of SPE compared with LLE are that it is faster, more reproducible, cleaner extracts are obtained, emulsion formation is not an issue, solvent consumption is reduced, and smaller sample sizes are required. It may also be cheaper than LLE when the costs for solvent disposal are taken into consideration. Moreover, SPE can be easily incorporated into automated analytical procedures. For a detailed introduction to SPE, the reader is directed to the relevant chapters in the Encyclopedia.

Solid phases currently employed for SPE include polystyrene divinylbenzene resins and chemically modified silica with either hydrophobic or ion-exchange groups. These materials generally yield satisfactory results if the conditions for extraction, washing and elution are carefully determined according to the chemical characteristics of the analytes and the other components in the sample. Nevertheless, due to the non-specificity of hydrophobic or ionic interactions, a large number of contaminants are often co-extracted, particularly if the sample matrix is very complex. A solution to this problem can be to use affinity sorbents, thus taking advantage of the specificity of biological recognition.

Theoretically, biomolecules like antibodies or receptors have the potential to meet the analytical demands for almost any target analyte since, with the advent of phage display antibody libraries and recombinant antibodies, a suitable recognition element can be found in many cases, even if a natural receptor does not exist. Unfortunately, biomolecules also have a major drawback, which is their poor stability, particularly in the presence of organic solvents. Artificial receptors have therefore been gaining in importance as a possible alternative to natural systems. Molecular imprinting is becoming increasingly recognized as a versatile technique for the preparation of synthetic polymers bearing tailor-made recognition sites. These molecularly imprinted polymers (MIPs) are obtained by copolymerizing functional and cross-linking monomers in the presence of the analyte, which acts as a molecular template. The molecular imprinting technique is reviewed in more detail elsewhere in the Encyclopedia (*see* Affinity Separation/Imprint Polymers). Molecularly imprinted polymers combine the advantages of biological antibodies or receptors (very specific binding) with those of synthetic polymers (high physical and chemical stability, compatibility with both aqueous and organic solvents). Their use in sample preconcentration and clean up by solid-phase extraction is therefore highly attractive.

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Analytical-Scale SPE

The applicability of imprinted polymers for SPE has been demonstrated on a number of compounds such as herbicides and drugs, which have been selectively extracted even from complex samples like beef liver extract, bile, blood serum and urine. **Figure 1** shows the principle of molecularly imprinted solid-phase extraction (MISPE). Typically, the sample is brought into contact with the imprinted polymer. This results in binding of the analyte and some impurities. Adsorption of the sample onto the polymer can be either from aqueous solution or from an organic or aqueous/organic solution after a solvent extraction step. The latter is often necessary to recover the analyte from solid or semi-solid samples, for example, soil or tissue. In both cases, the adsorption step is followed by a first elution step where impurities are washed away, whereas the specifically bound target analyte remains bound to the imprinted polymer. The last step is the elution of the analyte in concentrated and purified form using, for example, a competitor.

It has been shown that the target analyte can be recovered from samples at concentrations in the

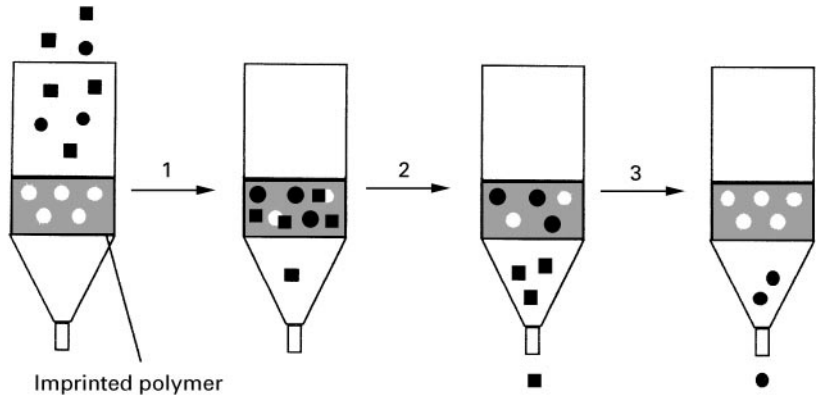

Figure 1 Principle of solid-phase extraction with an imprinted polymer. The sample is loaded onto the imprinted polymer, resulting in binding of both the analyte and some contaminants (step 1). A first elution step removes contaminants and the analyte remains in the specific binding sites (step 2). The analyte is then eluted from the polymer (step 3). ●, analyte; ■, other sample components.

lower $\mu g\,dm^{-3}$ to the $mg\,dm^{-3}$ range. The lower limit is determined by the dissociation constant of the analyte binding to the polymer. The upper limit is set by the binding capacity of the polymer. If the quantity of polymer used for extraction is increased, more analyte will bind, although non-specific binding of contaminants will also increase considerably. It is therefore necessary to optimize the extraction protocol for a specific application in terms of the amount of polymer used, the sample load, and the adsorption, washing and elution conditions, etc.

The quantification of the herbicide atrazine in beef liver is a good example of the utility of imprinted polymers in SPE. In a first step, atrazine was extracted from liver tissue with chloroform. The imprinted polymer was then used to clean the chloroform extract and to further concentrate the analyte prior to quantification. In this specific example, the binding capacity of the polymer for atrazine in chloroform was found to be 19 $\mu mol\,g^{-1}$. The analyte was eluted from the polymer with a suitable solvent (acetonitrile containing 10% acetic acid) and quantified, after drying and reconstitution in acetonitrile or buffer, by reversed-phase high performance liquid chromatography (RP-HPLC) or enzyme-linked immunosorbent assay (ELISA). When comparing the purified with the non-purified chloroform extracts in RP-HPLC, the SPE step with the imprinted polymer considerably improved the accuracy and precision of the HPLC method and lowered the detection limit from $20\,\mu g\,dm^{-3}$ to $5\,\mu g\,dm^{-3}$. This was achieved due to the removal of interfering components in the sample, resulting in baseline resolution of the atrazine peak. Furthermore, analyte recovery was increased from 60.9% to 88.7% (quantification by HPLC) and from 79.6 to 92.8% (quantification by ELISA).

Preparative-Scale SPE

What has been described thus far is, by and large, the use of molecular imprints as solid-phase extraction media in *analytical* applications, where the inherent selectivity of the imprints enables *efficient* clean up and/or preconcentration of samples prior to analyte quantification. Imprinted polymers are well suited to this purpose, and their functional capacity (the mass of analyte that can be bound per unit mass of polymer) places no undue restrictions on their widespread usage. In contrast, capacity is an important consideration in preparative-scale SPE, even if the solid-phase can be regenerated many times. Bearing in mind that the functional capacities of imprinted polymers prepared thus far have been moderately low, it is relatively easy to appreciate why only a few reports describing their potential use in preparative-scale work have appeared. However, there are certain niche applications that could conceivably be serviced by state-of-the-art materials where the low capacity is more than compensated for by the additional benefits that imprints confer, e.g. stability, selectivity, etc. In the long term it is certain that numerous opportunities will exist for the use of imprinted polymers in preparative-scale SPE once the low capacity issue has been addressed satisfactorily. They are well suited to product recovery from fermentation broths, production waste streams, and during chemical and enzymatic syntheses.

The term *facilitated chemical synthesis* in the context of preparative-scale SPE refers to chemical reactions that are performed and/or worked up in the presence of imprinted polymers. In the simplest case, this involves the addition of a polymer imprinted against the product to a vessel containing the crude reaction mixture. The imprint selectively binds the

product in preference to reactants, reagents, catalysts, etc., and is readily separated from the other components by virtue of its cross-linked insoluble character (the use of magnetic imprinted polymer particles or beads, which are available, would make this process even simpler). Alternatively, the crude reaction mixture can be passed through an appropriately sized SPE column, or the imprinted polymer can be placed in a product stream. Following selective adsorption, the product is washed out from the imprinted polymer and isolated. It can then be purified further if required. Another possibility for simple, inexpensive, and large-scale separations using imprinted-polymer particles is adsorptive bubble fractionation. The target compound is selectively adsorbed to the imprinted polymer in suspension within a cylindrical column containing a glass frit at the base of the column. Bubbles are formed when a gas is injected through the frit and they rise to the top of the column. Imprinted-polymer particles, which adhere to the gas bubbles, are transported to the top of the column where they accumulate and can then be easily recovered. It has been shown that enantiometric enrichment of L-phenylalanine anilide from a racemic solution can be achieved in this way, using an L-phenylalanine anilide-imprinted polymer. Overall then, imprinted polymers appear to offer an efficient general method for product isolation and purification, at least in principle.

Bearing in mind the functional capacity limitations associated with imprinted polymers at present, it should be clear why preparative-scale MISPE is currently not an attractive option. For the time being, it is more practical to use state-of-the-art imprinted materials in the selective extraction of impurities that are present in low or trace concentrations in crude reaction mixtures, where the functional capacities of imprinted polymers place considerably fewer restrictions on their application. In the following example, a recently reported model system focused on the chemical synthesis of the artificial sweetener α-aspartame (**Figure 2**). In the synthetic sequence, Z-protected L-aspartic acid anhydride (**2**) was reacted with L-phenylalanine methyl ester (**3**) to give Z-α-aspartame (**4**). The α-aspartame product (**1**) was obtained following removal of the Z-protecting group from the intermediate (**4**). The key feature about this reaction sequence was that a by-product, Z-β-aspartame (**5**), was formed during the first step, which then had to be removed. The typical composition of the crude

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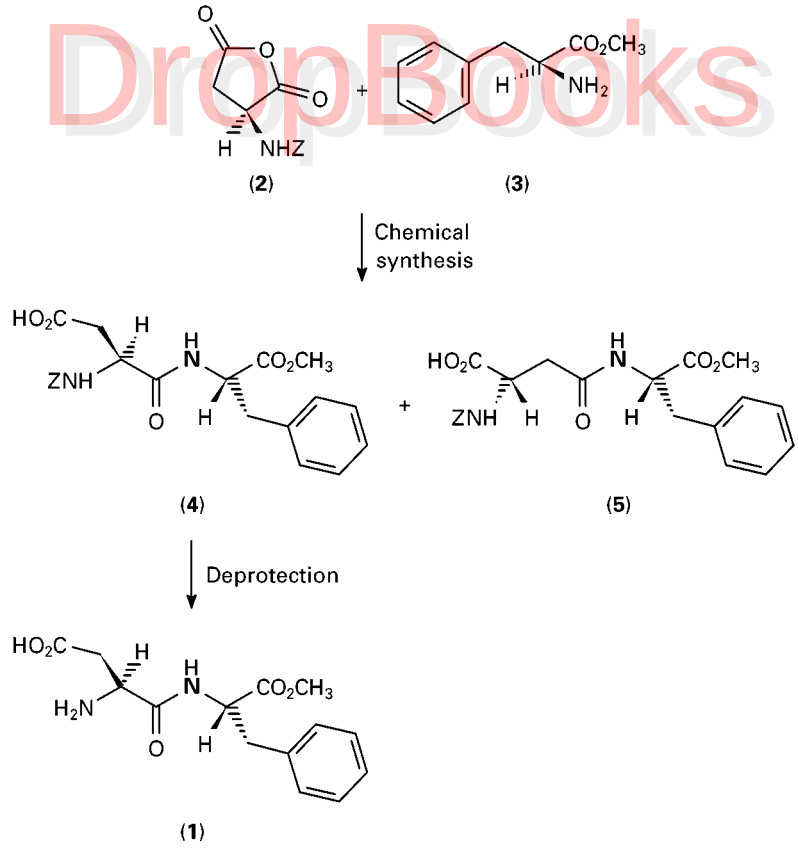


Figure 2 The chemical synthesis of α-aspartame. (1) α-aspartame, (2) Z-protected L-aspartic acid anhydride, (3) L-phenylalanine methyl ester, (4) Z-α-aspartame, (5) Z-β-aspartame.

reaction mixture was as follows: Z-α-aspartame, 59%; Z-β-aspartame, 19%; Z-L-aspartic acid, 22%. A polymer was imprinted against the by-product Z-β-aspartame (5) and used in the SPE mode for the selective removal of the by-product from the crude reaction mixture. After five passes through a solid-phase extraction column, the product purity was increased from 59 to 96%. In a control experiment using a nonimprinted polymer, the final purity achieved was only 86%.

It is also possible to use imprinted polymers advantageously *during* chemical reactions, to drive chemical equilibria in particular directions and thus influence product distributions in a controlled fashion. To prove this concept, the enzymatic condensation of Z-protected L-aspartic acid (6) with L-phenylalanine methyl ester (3) using the enzyme thermolysin was studied (see **Figure 3**). The equilibrium for this reaction, which normally lies to the left thus favouring reactants, can be driven to the right, in the direction of products by working in a nonsolvent for Z-α-aspartame. Another way of achieving the same effect would be to use a product-trap (product-sink) in the reaction itself, for example, a polymer imprinted against the product Z-α-aspartame. Indeed, when the reaction was carried out in the presence of a Z-α-aspartame-imprinted polymer, the reaction yield was found to increase from 15 to 63%.

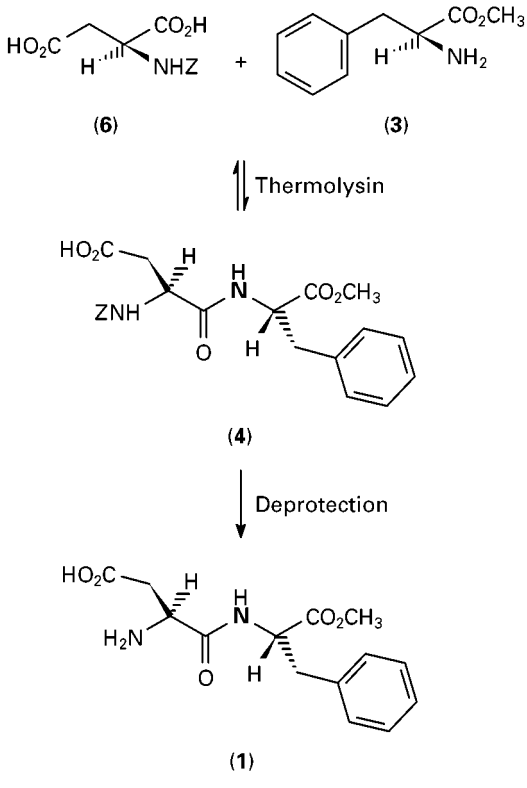


Figure 3 The enzymatic synthesis of α-aspartame. (1) α-aspartame, (3) L-phenylalanine methyl ester, (4) Z-α-aspartame, (6) Z-protected L-aspartic acid.

Present Limitations and Remedial Solutions

Any sorbent used in SPE must satisfy a range of performance criteria for a given application. Criteria that are of importance for imprinted polymers include: (1) Strong, selective, and reversible binding to the analyte; (2) fast mass-transfer kinetics; (3) high functional capacity; (4) minimal interaction of analyte with polymer backbone; (5) effective displacers available; (6) zero bleeding of template; (7) compatible with many solvents; (8) pressure resistant; (9) batch-to-batch reproducibility; (10) physical form of imprinted polymer; and (11) economics. Whilst imprints perform very well in many respects, there are certain limitations that need to be overcome in specific applications.

One of the most unsatisfactory features associated with the application of imprinted polymers as SPE sorbents in ultra-trace analysis is template leakage. Generally, once an imprinted polymer has been prepared, it is exhaustively extracted to remove the template from the polymer matrix. The difficulty in extracting 100% of the template molecule from an imprinted polymer has long been recognized, although until relatively recently it was widely believed that the few per cent of template remaining within the polymer was permanently entrapped. Recent work clearly demonstrates that this is not necessarily the case. What can and does occur is slow leakage of a portion of the remaining template from the polymer matrix over a period of time, even after exhaustive (solvent) extraction of the polymer beforehand. This template *leakage* or *bleeding* can have serious implications when the polymer is being used as an SPE sorbent in ultra-trace analysis, although is of much less concern in trace analysis.

Whilst a general solution to the bleeding problem is being sought by researchers across the globe, a possible method of circumventing the bleeding problem entirely is to use a template analogue during the imprinting step, rather than the template itself. One of the first demonstrations of this approach was described by Andersson *et al*. in a paper detailing the use of MISPE for the preconcentration of the drug sameridine (7) in human plasma, prior to its quantification via gas chromatography (GC). At the nanomolar concentration levels used in the study, leakage of template from the polymer matrix during sample handling was considerable and easily detectable via GC analysis. Bearing in mind the application and the concentration window, such leakage was completely

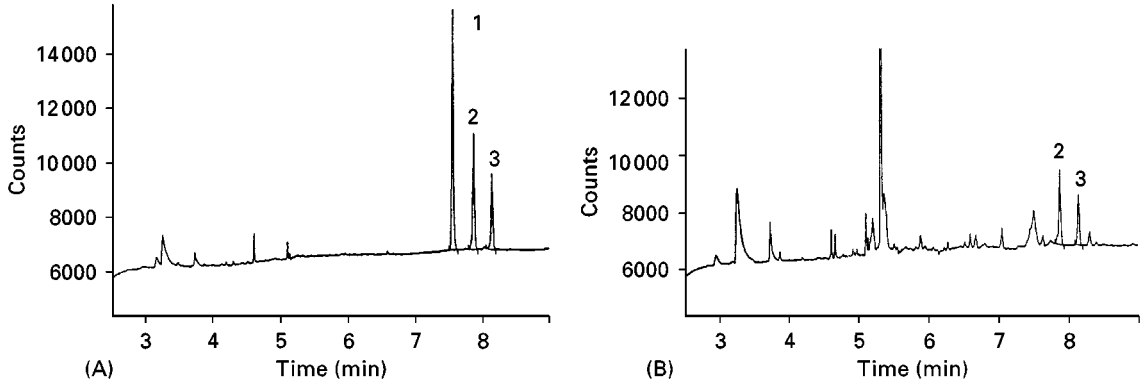
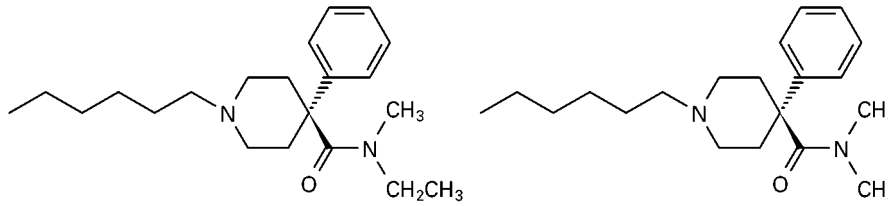


Figure 4 GC traces of human plasma samples spiked with sameridine and an internal standard, and subjected to (A) solid-phase extraction with an imprinted polymer and (B) standard liquid–liquid extraction. The peaks are (1) the template molecule (a close structural analogue of sameridine), (2) the analyte sameridine and (3) the internal standard. (Reproduced with permission from Andersson, Papricia and Arvidsson, 1997).



unacceptable because it led to large errors in the precision of the analytical measurement. Rather than take steps to minimize or eradicate leakage, a close structural analogue of sameridine (8) was used as the template molecule in the imprinting step, which yielded an imprinted polymer that still displayed a strong affinity for sameridine.

Following solid-phase extraction of sameridine from human plasma using this polymer, leakage of the analogue from the polymer matrix did occur, but sameridine and the analogue were readily resolved using GC and the sameridine was subsequently quantified. The results obtained were as good as those obtained via a standard liquid–liquid extraction method, with the added advantage that the plasma sample contained fewer matrix contaminants with MISPE than for LLE, i.e. the sample for assay was much cleaner (**Figure 4**).

The template-analogue method does rely upon the availability of a close structural analogue of the analyte, and also a strong affinity between the polymer imprinted against the analogue and the analyte. One or both of these criteria may not always be fulfilled, therefore new approaches are required to tackle the bleeding issue.

In a solid-phase extraction operation, one ideally wants strong binding of the analyte to the sorbent during the loading and washing steps, and rapid stripping of the analyte from the sorbent during the elution step, ideally in as small a volume as possible. Efficient loading and washing is therefore favoured by a strong affinity between the polymer and the analyte, whereas efficient elution is obtained when the affinity is moderate to weak. In some cases, MIPs can actually bind analytes too strongly, which means that stronger displacers or larger volumes of eluting solvent are required than would otherwise be considered ideal. In some circumstances, therefore, having an MIP of lower affinity but with the same selectivity would be desirable. One way of achieving this goal might be via thermal pretreatment of the polymer at high temperature prior to use, which can have the effect of killing off a proportion of the high-energy binding sites.

As indicated already, the low functional capacity of imprinted polymers does not unduly limit their potential in analytical applications, although it does place restrictions on their immediate value in preparative-scale solid-phase extractions. With the advent of new developments in the imprinting area, and the advantageous knock-on effect this is likely to have on capacities, it is expected that preparative-scale applications will become more feasible in the future. Scale-up of the polymerization process to an industrial scale must also be addressed. In future developments, the elaboration of imprinting methodologies in polar environments is also expected; at present, it is a challenge to prepare good imprints in these

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'competitive' environments. Although it is possible to imprint certain templates in polar environments, polar solvents (e.g. water) tend to interfere to an unacceptable level with the non-covalent interactions between template and functional monomer that are often relied upon in imprinting protocols. What is more common and easier to deal with, however, is the use of imprints effectively in buffer and/or polar environments. Finally, the imprinting of larger templates (e.g. proteins) is rather challenging at present due to their 'fragile' nature. New synthetic developments should lead to progress in this area also.

Conclusions

Molecularly imprinted polymers constitute a new class of sorbents which combine the robust character of cross-linked polymers with the attractive properties of natural receptors. In sample clean up and concentration for trace and ultra-trace analysis, they offer distinct advantages over both liquid–liquid extraction and solid-phase extraction using classical sorbents and immunosorbents. Besides analytical applications, imprinted polymers are being increasingly considered for preparative-scale SPE applications, even though their present low functional capacity sets a limit on their widespread utility. However, concomitant with improvements in their capacity, it will become increasingly appealing to use imprinted polymers to remove products and/or by-products from reaction vessels/streams, and to influence directly the course of chemical reactions by 'equilibrium shifting'. Finally, it is worth noting that the area of molecular imprinting as a whole is undergoing rapid expansion at present. What this implies for MISPE is that one can expect tailored, high-performance imprinted polymers to become increasingly attractive and more widely available as methodologies improve and breakthroughs are made.

See also: **II/Extraction:** Solid-Phase Extraction. **III/Immunoaffinity Extraction. Selectivity of Imprinted Polymers: Affinity Separation:**

Further Reading

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MOLECULAR RECOGNITION TECHNOLOGY IN INORGANIC EXTRACTION

J. D. Glennon, University College Cork, Cork, Ireland

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Selective ion recognition, binding and transport are important processes in living systems. From the active sites of metalloproteins, such as amine oxidase, to lower molecular weight ligands like valinomycin, the K^+-selective macrocyclic antibiotic, the underlying principles of such selective ion binding and utilization have for some time been the subject of intensive investigation and modelling by many researchers.